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The essential oil of Algerian *Ephedra alata* subsp. *alenda* and its antimicrobial properties

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ABSTRACT

Ephedra, a medicinal plant belonging to the Ephedraceae Dum. Family, is a genus of non-flowering seed plants belonging to the Gnetales, the closest living relative of the Angiosperms. These *Ephedra* species have medicinal, ecological, and economic value. The aim of this study was to conduct a chemical characterization of essential oil was obtained by hydrodistillation and analyzed by gas chromatography–mass spectrometry. The essential oil from the aerial parts of *Ephedra alata* was rich in B pinene (42.57%), α-Terpinyl acetate(28.85%) β-Selinene (10.88%) Borneol(7.56%) β-Cadinene (4.23%). Their antibacterial effects towards *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes* were tested by a disc diffusion assay. Essential oil of *Ephedra alata* extracted by hydrodistillation had the highest efficiency against three positive strains of bacteria (*S. aureus*, *B. cereus* and *L. monocytogenes*).

Key words: *Ephedra alata* , antibacterial activity, GC/SM Essential oil, Essential oil.

INTRODUCTION

From the ancient days, medicinal plants have been a valuable source of various therapeutics, and still represent an essential fund of new plant-derived natural products or their derivatives with significant biological activities (Abu Khalaf 2016; Atanasov et al. 2015). Ephedra alata belongs to the Ephedraceae family. It is a species of Ephedra that grows mostly in the desert. genus *Ephedra* contains more than 60 species of non flowering seed plants distributed throughout Asia, America, Europe, and North Africa. (Poli et al. 2018) IT is a perennial tough shrubs, with yellow-green and 50-100 cm tall twigs Ephedra species have a long history in traditional Chinese medicine. This species showed anti-inflammatory, anticancer, antibacterial, antioxidant, hepatoprotective, anti-obesity, antiviral, and diuretic activities. It is uses in the treatment of allergies, nasal congestion, bronchial asthma, coughs and flu (White et al.1996). These studies have emphasized the existence of marked chemical differences among oils extracted from different species or varieties. These variations are likely to influence the antimicrobial activity of the oil and are generally a function of three factors: genetically determined properties, the age of the plant and the (Chouitah et al. 2017) .The aim of this study was to conduct a chemical characterization of the essential oil of the aerial part *Ephedra alata*., which is indigenous to Algeria and the antimicrobial activity.

MATERIALS AND METHODS

Plant material

Plant materials were collected in Ain Sefra North of Algeria in May 2018. A voucher specimen is deposited in the herbarium of the Department of Ecology at the Agronomic Institute.

The fresh aerial parts of *Ephedra alata* were dried in room temperature for five days. Then, the dried material (100 g) was mixed with 2.5 L of distilled water and subjected to hydrodistillation in a Clevenger-type apparatus for 3 h to afford a pale yellow oil. The isolated oil, after drying over anhydrous sodium sulfate (Na_2SO_4) and filtration, was stored in sealed glass vials and maintained under refrigeration until further analysis. Total oil yield was expressed as a percentage (g per 100 g of dried leaves). Analyses were replicated three times.

Gas-Chromatography - Mass Spectrometry analysis of essential 0il

Qualitative analysis of the chemical composition of the essential oil was performed with a gas chromatograph (GC) coupled with a mass spectrometer (MS), Agilent Model GC-7890B/MSD-5977A (quadrupole), with an electron impact at 70 eV, an HP-5MS methylpolysiloxane column (30 m × 0.25 mm × 0.25 µm, Agilent), a 1 mL/min flowing helium carrier gas, an injector temperature of 250 ◦C, a detector temperature of 150°C. Injector type 1177 was heated to a temperature of 220 ◦C. Injection mode was splitless (1 µL of a 1:1000 n-hexane solution). Helium was used as a carrier gas at a constant column flow rate of 1.2 mL/min. Column temperature was programmed-initial temperature was 50 °C for 10 min, then increased to 100 °C at 3°C/min, was maintained as isothermal for 5 min, and then increased to 150 °C at 10 °C/min. The total time for analysis was 87.67 min. Analyses were also run with the same operating conditions by using an apolar DB-5 fused silica capillary column (30 m×0.25 mm, 0.25 µm film thicknesses). In both cases, helium was used as the carrier gas (1.2 mL/min). The mass spectrometer trap was heated to 200 °C, manifold 50°C, and transfer line 270 °C. The GC-MS system was equipped with a TR-5 MS column (30 m × 0.32 mmi.d., 0.25-µm film thickness, THERMO Scientific Corp.) Rubini et al. (2018) carried out an analyses using helium as a carrier gas at a flow rate of 1.0 mL min^{-1} and a split ratio of 1:10 using the following temperature program: 60 °C for one minute; rising at 4.0°C min^{-1} to 240 °C, and heldMolecules 2019, 24, 584 9 of 12for one minute. The injector and detector were held at 210 °C. Diluted samples (1:10 hexane, v/v) of one µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. The various constituents of the EO were identified by visually comparing their mass spectra with those in the literature.

Analysis of the essential oils

The Physical and chemical properties such as color, refractive index, density, freezing point, rotational power, acid index and ester index were observed. The essential oil refractive index was determined using the Abbe refractometer. To determine the freezing point, a small amount of oil was placed in a capillary with a standard thermometer. The density of the essential oil was determined using the densitometer bottle. If optical rotation was determined, with polar meter the direction of rotation was determined.

Microbial strains

Bacterial Strains and Culture Conditions EOOG activity was evaluated against two representatives, Gram-negative and -positive bacterial cells, four strains of S. aureus and E. coli species. The standard strains from the American Type Culture Collection (ATCC, USA) were used as sensitive antibiotic strains (Oliva et al. 2018). The clinical S. aureus were isolated and identified in the Laboratory of Microbiology, as part of the hospital routine (Fadli et al. 2012) .The antibacterial test of the studied EO was carried out using disc diffusion method. tested in vitro against: *Escherichia coli Pseudomonas aeruginosa* as Gram-negative bacteria and *Staphylococcus Aureus Bacillus cereus* as Gram positive bacteria suspensions were adjusted to1×107 CFUmL−1 (equivalent to 0.5 McFarland). Antimicrobial tests were carried out using the disc diffusion Method. Essential oil was dissolved in dimethyl sulphoxide (DMSO) to obtain different concentrations as follow 6.25, 12.5, 25, 50 and 100 µg/mL.

Determination of Minimum Inhibitory Concentration (96-Well Microplate Method) he antibacterial effect was evaluated by measuring the diameter of inhibitory zones (DIZ) in millimeters, and the results were expressed as means from three determinations. A 4 mL liquid suspension from fresh fungal cultures (96 h) was prepared at 108 spore/mL. The tested EO was dissolved in potato dextrose broth (PDB) at 800, 1000, 1200, 1400, and 1600 µL/mL according to the obtained results from the initial screening assay (Tangpao 2018)Two hundred µL/well from each prepared concentration of EO and 100 µL/well of the prepared suspension were added in the microplate and then incubated at 24±2◦C. The absorbance was measured at λ =450nm using an Elisamicroplate reade rinstrument (DASs.r.l., Rome, Italy) after 48, 120, and 168 h (Palaniappan 2018). The assay was carried at three times and any zone of inhibition listed as positive results.

RESULTS AND DISCUSSION

The yield of essential oils of the air-dried aerial parts of the three species investigated were 1.79% of dry weight. Essential oils soluble in organic solvents insoluble in water and colorless slightly

yellowish color with aromatic flavor . Determination of density was done by double weighing d = 0. 87, the refractive index n =1.4555 and the Specific rotation = +0.25 by polarimetry .In the studied EO, a high percentage of compounds, around 99%, were identified. The chemical composition profile included a broad spectrum of components, such as monoterpene derivatives. The main compound of ESSENTIAL oil from the aerial parts of *Ephedra alata* was rich in B pinene (42.57%), α-Terpinyl acetate(28.85) β-Selinene (10.88) Borneol(7.56) β-Cadinene (4.23%). The amounts of the other nine components were in the range of 1–3.9%. Composition of EO depends on ecological and climatic conditions, the ontogenesis phase, as well as from the processing within the harvest and method of isolation, and generally the yield of EO increase with plant maturation Almeida et al. (2017) referring to the large inhibition zones observed with disc diffusion method, were determined for 3 very sensitive bacterial strains that showed a diameter of inhibition above 16 mm and the results are reported in Table 3. The lowest MIC were 5 ,10 µL/mL was noticed against *S. aureus*, and *B. cereus*. This activity could be related to the amounts of oxygenated mono-and sesquiterpene hydrocarbons (Parsaeimehr et al. 2010) and especially to the high percentage of B pinene present in the oil. However, it is possible that the minor compounds give rise to the antibacterial activity after 24h of exposure to the essential oil for all bacterial strains tested. The interesting activity of the essential oil on several bacteria made it necessary to find the components responsible for this effect. The essential oil of showed an interesting antimicrobial effect for some bacteria *(Escherichia coli, Staphylococcus aureus, Bacillus cereus and Listeria monocytogenes).* This essential oil has highlighted the appreciable antimicrobial capacity .their effectiveness varied with concentration, type of the essential oils and the type of bacteria species. Overall, all essential oils had overriding antibacterial effect against gram positive and gram negative bacteria.

Table 1. Physicochemical composition of *Ephedra alata.*

Specification	Density D	Refractive index	Optical activity N20	Solubility in ethanol 90%	Freezing point °C
Ephedra alata	0.87	1.4555	0.25	1:3	-18

Table 2. The major identified components in essential oil from *Ephedra alata* analyzed by GC-MS technique with retention indices on HP-5MS capillary column.

Volatile compounds	RI Apolar Column	RI Polar Column	Area %
α-thujene	921	939.5	2.6
Camphene	940	946	1.25
Sabinene	966	969	3.5
B pinene	969	974	42.57
-Terpinene	1014	1018	0.22
Limonene	1029	1235	17.66
α-Terpinyl acetate	1165	1161	28.85
Borneol	1258	1266	7.56
bornyl acetate	1285		00.5
Germacrene	1492	1455	06.50
β-Selinene	1455.8	1492	10.88
6 β-Guaiene	1458.6	1513	0.2
β-Cadinene	1483.8	1521	4.23
Trans-calamenene	1488.8	1513	0.12
α-Calacorene	1550	1566	1.97
spathulenol	1578	1590	0.10
Globulol	1584	1588	0.19
Farnesol	1592	1634	0.30
Total			**99%**

a= retention indices on MetSil column; b= retention indices on CP-Sil 88 column.

Table 3. Inhibition zone (mm) using direct contact technique in agar medium and MIC (g/mL) for the essential oil using microdilution method in 96 multiwall microliter plate.

Microorganisms	**Strain**	**Diameter of inhibition**	**MIC g/ml**
Staphylococcus aureus	ATCC 29230	10±0.1	05.00
Escherichia coli	ATCC 4350	17.3±0.10	16.0
Listeria monocytogenes	ATCC25352.1	11.50±0.03	09.00
Bacillus cereus	ATCC 170885	12 ±0.02	10.00

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